Recombinant Human Interleukin-8 Is a Potent Activator of Canine Neutrophil Aggregation, Migration, and Leukotriene B_4 Biosynthesis

M. Krogsgaard Thomsen, C. Gronhøj Larsen, H. Klem Thomsen, D. Kirstein, T. Skak-Nielsen, I. Ahnfelt-Rønne, and K. Thestrup-Pedersen

Department of Pharmacology (MKT, DK, TS-N, IA-R), Leo Pharmaceutical Products, Ballerup; Department of Dermatology (CGL, KT-P), Marselisborg Hospital, University of Aarhus; and Department of Pathology (HKT), Bispebjerg Hospital, Copenhagen, Denmark

Interleukin-8 (IL-8), formerly known as NAP-1, is formed by a variety of cells upon stimulation with IL-1 or tumor necrosis factor (TNF). The biologic activity of the cytokine involves activation of almost every neutrophil function studied so far in different species. In the present study, we compared the effects of recombinant human IL-8 (rIL-8) and the lipid mediators, leukotriene B_4 (LTB_4) and platelet-activating factor (PAF), on neutrophil functions in dogs. All three chemotactic factors induced neutrophil aggregation and chemotaxis, with rIL-8 being far more potent than LTB_4 and PAF. The migration induced by rIL-8 was significantly greater than that observed towards LTB_4 and PAF. In the aggregation assay, rIL-8 was shown for the first time to be a potent stimulant. The aggregation response was more persistent than that obtained with LTB_4 and PAF and the potency of rIL-8 was greater. An intradermal dose-response study showed that rIL-8 is an extremely potent inducer of selective neutrophil infiltration in canine skin. The infiltration was more pronounced than following injection of LTB_4 or PAF. It was proposed that the superior effect of rIL-8 was caused by a synergistic effect between injected rIL-8 and LTB_4, which was shown to be produced in biologically active amounts by canine neutrophils stimulated with rIL-8. From a therapeutic point of view, the simultaneous presence of rIL-8 and LTB_4 in inflammatory skin diseases highlights the need to develop drugs that inhibit the production and/or effect of both mediators. *J Invest Dermatol 96:260–266, 1991*

Until recently, human interleukin-1 (IL-1) was believed to be a stimulant of neutrophil migration in vitro [1–2]. However, highly purified natural IL-1 as well as rIL-1 proved unable to stimulate the migration of human neutrophils in vitro [3] and it only acts as a weak chemoattractant for canine neutrophils [4]. Furthermore, IL-1 activity in the conditioned medium of LPS-stimulated monocytes could be separated from the activity of a novel 8-kD neutrophil chemotactic peptide, subsequently purified and molecularly cloned [3,5]. Because we additionally established the potent T-lymphocyte chemotactic activity of this factor, it is now termed IL-8 [6]. IL-8 is inducible in monocytes, keratinocytes, dermal fibroblasts, and endothelial cells when stimulated with IL-1 or tumor necrosis factor (TNF) [6] and, apart from promoting neutrophil migration in vitro and in vivo, IL-8 also exerts other biologic effects on neutrophils. Thus, IL-8 induces enhanced release of lysosomal enzymes and activates the respiratory burst leading to generation of superoxide in neutrophils [7–9]. Also, human neutrophils and IL-3 primed basophils stimulated with IL-8 have been shown to generate leukotriene B_4 (LTB_4) and LTC_4, respectively, due to activation of the 5-lipoxygenase enzyme [10,11]. This indicates that IL-8 promotes some of the effects formerly assigned to IL-1 and TNF [12]. IL-8 is now believed to be an important pro-inflammatory mediator involved in the pathogenesis of neutrophil-dominated human inflammatory diseases such as psoriasis [13] and rheumatoid arthritis [14]. Human rIL-8 provokes a neutrophil infiltrate when administered to humans [15] but has been found also to activate neutrophils from other species, including the mouse, guinea pig, rat, and rabbit [12,16,17].

In the present study, we compared the in vitro and in vivo activity of rIL-8 and the lipid mediators, LTB_4 and PAF, on canine neutrophil aggregation and migration. Further, we studied the effect of IL-8 on 5-lipoxygenase-catalyzed arachidonic acid (AA) metabolism in canine neutrophils.

Manuscript received May 16, 1990; accepted for publication October 18, 1990.

Reprint requests to: M. K. Thomsen, Department of Pharmacology, Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark.

Abbreviations:
AA: arachidonic acid
BSA: bovine serum albumin
HBSS: Hank's balanced salt solution
IL-1 (-8): interleukin-1 (-8)
LTB_4: leukotriene B_4
PAF: platelet-activating factor
r: recombinant human
RC-HPLC: reverse-phase high-performance liquid chromatography
TNF: tumor necrosis factor

MATERIALS AND METHODS

Animals Healthy Beagle dogs that had not received any drug or test compound the preceding two weeks were used. For chemotaxis, LTB_4 production, and aggregation experiments 20, 60, and 100 ml of EDTA-stabilized venous blood was obtained, respectively. Experimental, intradermal injections of rIL-1α, rIL-8, LTB_4, and PAF were performed on the dorsum of dogs that had been shaved 2 h earlier.

Isolation of Neutrophils Neutrophils with a purity of at least 95% were isolated as previously described [18]. Briefly, red blood cells were allowed to sediment for 45 min on methylcellulose, fol-

0022-202X/91/$03.50

lowed by centrifugation of leukocyte-rich plasma on Lymphoprep (Nycomed, Norway) for 30 min at 400 × *g*. The resulting neutrophil-containing cell pellet was then washed twice in Hank's balanced salt solution (HBSS) (Gibco, Grand Island, NY) containing 0.5% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO) and the neutrophil suspension was adjusted to 2×10^6 cells per ml. Cell recovery was between 40 and 50%, and viability was approximately 97% as determined by the Eosin Y exclusion test.

Chemotaxis Assay Cell migration was studied in a 48-well chemotaxis microchamber (Neuroprobe Inc., Bethesda, MD) using 10 μm thick, 3-μm–pore polyvinyl pyrrolidone-free polycarbonate filters (Nucleopore Corp., Pleasanton, CA) [19]. Twenty-five microliters prewarmed LTB_4 (Ultrafine Chemicals, UK), PAF (Sigma), or rIL-8 [17] diluted in HBSS containing 0.5% BSA was placed in the lower well and 50 μl cell suspension (2×10^6 cells per ml) was placed in the upper chamber. Incubation proceeded for 45 min at 37°, 5% CO_2, and 98% humidity. Following this, filters were fixed and stained with Coumassie brilliant blue (Sigma). Migration was estimated by lower surface counting. The mean of the medians of five individual readings on each of duplicate filters was calculated and the results were expressed as chemotactic differentials.

Aggregation Assay Cells were isolated using divalent cation-free HBSS and adjusted to 3×10^7 cells per ml. Aggregation was assayed as described previously [18]. Briefly, 200 μl neutrophil suspension, 5 μl recalcification medium (40 mM $CaCl_2$ and 30 mM $MgCl_2$) was placed in a siliconized aggregometer cuvette and aggregation was induced by adding rIL-8, LTB_4, or PAF, once the baseline was stable. The Payton aggregometer was set at 37°, 900 rpm, with 0 and 100% light transmission representing transmission through the cell suspension and a cell suspension to which 10% HBSS was added, respectively. Light transmission was recorded for 5 min and the extent of aggregation was expressed as the percentage increase in light transmission. All experiments were carried out in duplicate.

Phagocytosis A visual *Candida albicans* blastospore ingestion assay was used as previously described [4].

rIL-8 Induced LTB_4 Production Suspensions of 10^7 purified neutrophils in 1 ml divalent cation-free HBSS were preincubated with 8 μM AA (Sigma) or 1 μCi [1-^{14}C]AA (specific activity, 54.2 mCi per mmol; New England Nuclear, FRG) for 5 min (37°) before stimulation (5 min at 37°) with 100 nM rIL-8 in a final volume of 1.6 ml Ca^{++}/Mg^{++}-enriched HBSS [10]. After cooling and centrifugation at 4°, the supernatants were freeze-dried and deproteinized with 200 μl eluent for reverse-phase high-performance liquid chromatography (RP-HPLC): 70% methanol, 30% water, 0.08% acetic acid, adjusted to an apparent pH of 6.2 with NH_4OH. Samples of 100 μl were applied to a Merck LiChrospher 100 RP-18 (125 × 4 mm, 5 μm) column and analytes were eluted at a flow rate of 1 ml per min. LTB_4 was detected at 270 nm by comparing the retention time with that of synthetic LTB_4.

In Vivo Experiments rIL-8 was diluted to a final concentration of 0.5, 5, or 10 nM in phosphate-buffered saline and injected intradermally in duplicate in a volume of 100 μl. At 2, 4, and 6 h after injection, the skin sites were infiltrated with 0.5% lidocaine (Leostesin, Leo Pharmaceutical Products, Denmark) subcutaneously, and a 4-mm punch biopsy (Stiefel, Belgium) was taken. Further intradermal experiments were carried out with varying concentrations of rIL-1α (Boehringer Mannheim, FRG), LTB_4, and PAF. Because IL-1 [20], but not IL-8 [21], induces biosynthesis of proteins in various cells, rIL-1α was administered alone or in combination with a protein biosynthesis inhibitive dose [20], 0.1 μmol, of cycloheximide (Sigma).

Histopathology The biopsies were processed by conventional techniques, and histologic preparations were stained with haematoxylin-eosin. All experiments were blinded to the observer, carried out in duplicate, and corrected for solvent controls in each case. The presence of inflammatory cells was quantified by assigning a score of 1, 2, and 3 to skin sections with mild, modest, and pronounced dermal neutrophil infiltration, respectively, as previously described [22].

Myeloperoxidase Determination Quantitative comparison of the neutrophil infiltrate occurring in response to rIL-8, LTB_4, and PAF was performed by determining the skin content of the specific neutrophil enzyme marker, myeloperoxidase, as previously reported [23]. Briefly, duplicate skin biopsies were homogenized in a hexadecyltrimethylammonium-bromide (Sigma) buffer, and the homogenate was freeze/thawed 3 times, followed by centrifugation at 2,500 × g for 15 min (4°C). The supernatant was incubated for 10 min with a tetramethylbenzidine/H_2O_2 solution, and the absorbance at 655 nm was measured. The results were expressed as the number of neutrophils per cm² skin, as determined from a simultaneously obtained standard curve for purified blood neutrophil suspensions.

Statistical Methods Student t test for paired samples was used for comparison of in vitro and in vivo data.

RESULTS

Chemotaxis Dose-response relationships for induction of canine and human neutrophil chemotaxis were obtained for rIL-8, LTB_4, and PAF by the lower surface count technique. All three chemoattractants were capable of inducing dose-dependent chemotaxis in cells from both species. The only significant ($p < 0.05$) difference found regarding the maximal response (E_{max}) to the mediators was that rIL-8 was more active towards canine cells than LTB_4 and PAF (Fig 1). In both species, however, the molar potency of rIL-8 was by far the greatest (Table I).

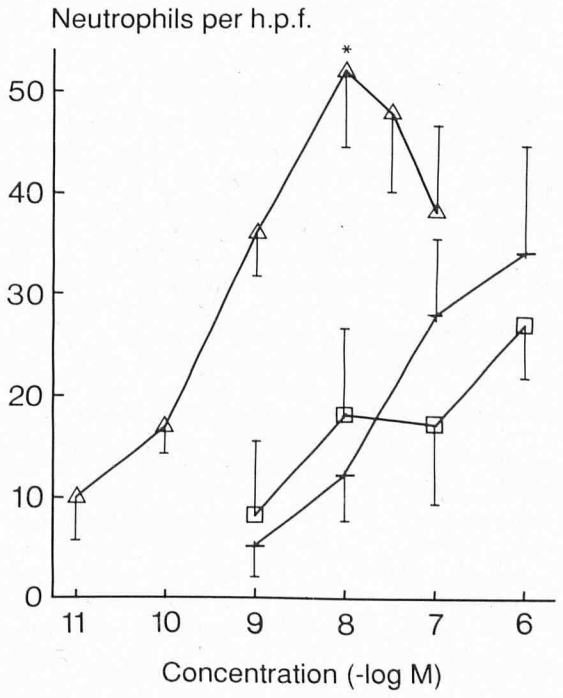

Figure 1. Effect of varying concentrations of IL-8 (△), LTB_4 (+), and PAF (□) on canine neutrophil migration. Cells were allowed to migrate for 45 min (37°, 5% CO_2) towards the chemoattractant, and the locomotory response was corrected for random migration, i.e., migration in the absence of chemoattractant. Results are presented as the means ± SE of four individual experiments, each experiment including assay of all three chemoattractants on cells from one dog. *, $p < 0.05$ as compared to maximal migration towards LTB_4 and PAF.

Table I. Potency (EC_{50}) and Efficacy (E_{max}) of IL-8, LTB_4, and PAF in Canine and Human Migration and Aggregation Assays

Cells	Assay	Mediator	EC_{50}[a]	E_{max}[b]
Canine	Migration	IL-8	0.2	52
Canine	Migration	LTB_4	23	34
Canine	Migration	PAF	123	27
Human	Migration	IL-8	0.1	28
Human	Migration	LTB_4	2	30
Human	Migration	PAF	110	25
Canine	Aggregat.	IL-8	0.1	57
Canine	Aggregat.	LTB_4	8	62
Canine	Aggregat.	PAF	32	63
Human	Aggregat.	IL-8	0.1	48
Human	Aggregat.	LTB_4	3	56
Human	Aggregat.	PAF	57	51

[a] nM.
[b] Cells per high-power field.

Aggregation rIL-8, LTB_4, and PAF all induced aggregation of canine neutrophils in a dose-dependent manner (Fig 2). The onset of aggregation induced by rIL-8 was relatively slow when compared to LTB_4 and PAF-induced aggregation (Fig 3). However, cell aggregation following stimulation with IL-8 was more persistent than following stimulation with LTB_4 or PAF (Fig 3). Using both canine and human neutrophils, the dose-response curve for rIL-8 was a factor 30 to 600 to the left of the curves for LTB_4 and PAF (Table I), in accord with the potency ratios (ratio between concentrations of two different mediators evoking the same biological response) in the chemotaxis assay.

Phagocytosis At optimal serum opsonin (C_{3b}) concentrations, no effect of rIL-8, LTB_4, or PAF on phagocytosis was seen (data not shown).

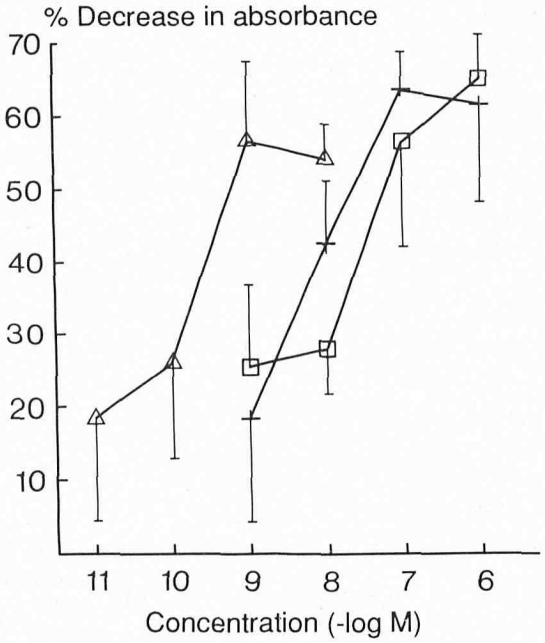

Figure 2. The peak aggregatory response of canine neutrophils to varying concentrations of rIL-8 (△), LTB_4 (+), and PAF (□). Light transmission was measured for 5 min and peak aggregation was measured as percentage increase in light transmission. The experiments were performed at 37°C and 900 rpm. Results are shown as the means ± SE of three individuals experiments, each experiment assessing the effect of all three mediators on cells from one dog.

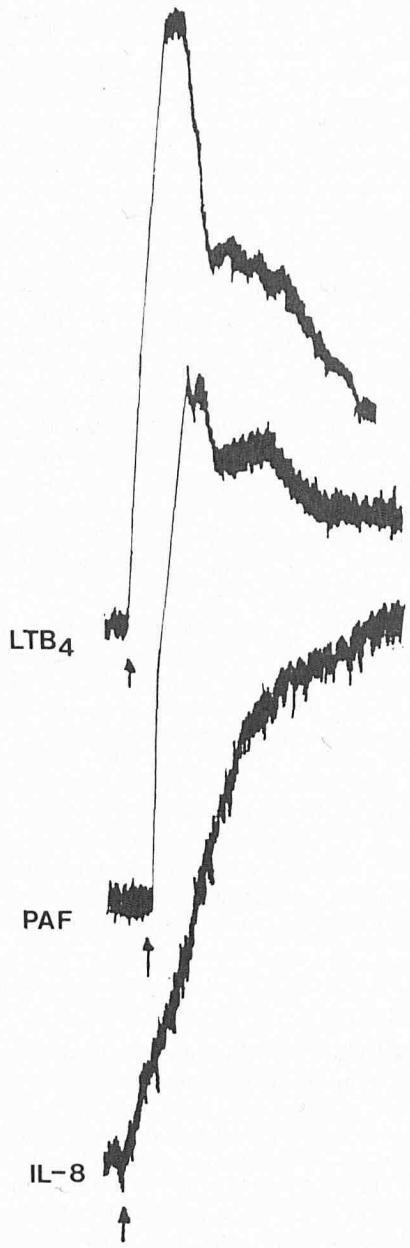

Figure 3. Typical aggregation pattern obtained with rIL-8 (1 nM), LTB_4 (100 nM), and PAF (100 nM) in canine neutrophils. The stimulant was added as indicated by *arrows*. The percentage increase in light transmission is shown.

rIL-8–Induced Production of LTB_4 by Canine Neutrophils Incubation of purified canine neutrophils with 100 nM rIL-8 in the presence of 8 μM exogenous AA resulted in the production of substantial amounts of LTB_4 (up to more than 100 ng/10^7 cells) and other UV-absorbing peaks, as shown in a typical chromatogram (Fig 4*A*). When neutrophils were incubated with AA in the absence of IL-8, only trace amounts of LTB_4 could be detected (Fig 4*B*). The identification of LTB_4 was based on co-elution with authentic LTB_4 (Fig 4, *inset*) and comparison of chemotactic activity of the peak eluting at 6.9 min with that of authentic LTB_4. For this peak, the EC_{50} (concentration evoking half-maximal response) for induction of chemotaxis was found to be 9 ng per ml, which is similar to the EC_{50} of 7 ng per ml reported for authentic LTB_4 in the chemotaxis section. The peaks eluting earlier than LTB_4 represented chemotactically inert products of AA metabolism, as shown by assay of migration and experiments with ^{14}C-labeled AA in which peaks of

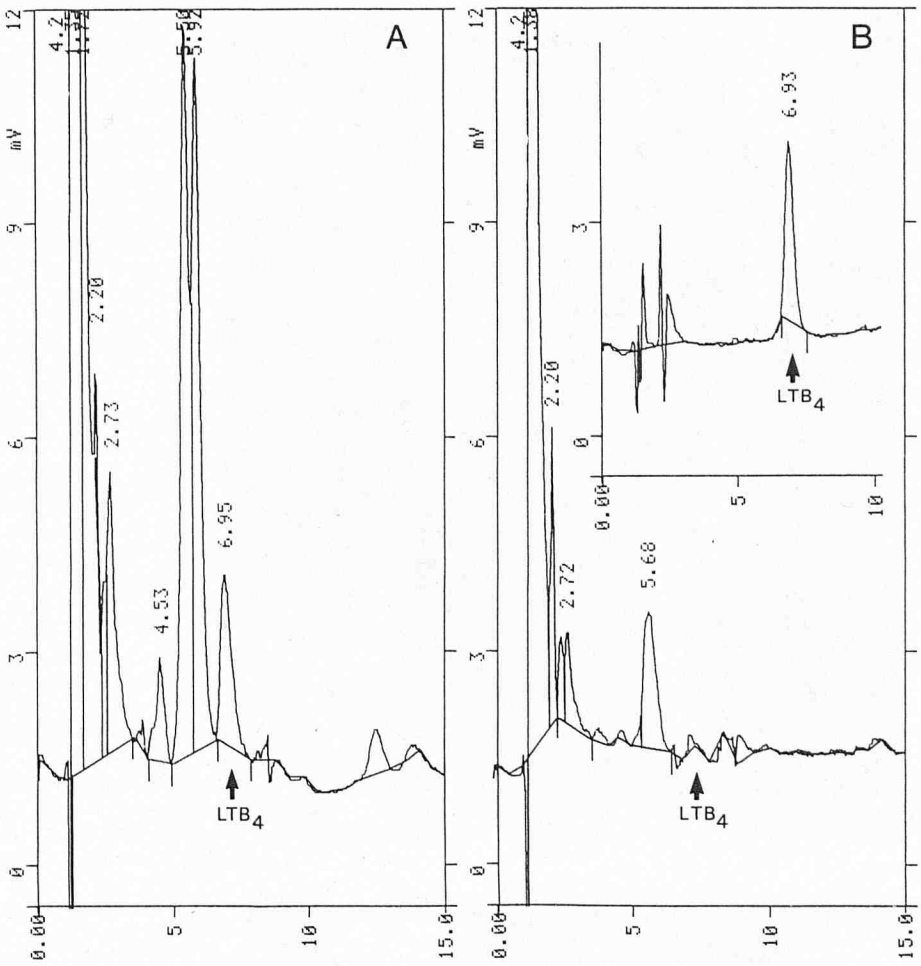

Figure 4. RP-HPLC chromatograms showing the production of LTB_4 by rIL-8 stimulated neutrophils. 10^7 cells were preincubated with 8 μM AA and incubated with 100 nM rIL-8 *(A)* or buffer *(B)* in the presence of Ca^{++} and Mg^{++}. The *inset* shows the elution of 32 ng synthetic LTB_4. Abscissa, min; ordinate, mV corresponding to milliabsorbance. A typical chromatogram out of three individual experiments with different dogs is shown.

radioactivity co-eluted with the peaks shown in Fig 4*A*. By analogy with the similar elution profile obtained in a previous study with human cells [10], the early peaks may represent 20-COOH-LTB_4, 20-OH-LTB_4, trans-LTB_4, and epi-trans-LTB_4.

In Vivo Experiments Intradermal injection of rIL-1α in dogs did not provoke any macroscopic changes. Microscopically, a modest but selective dose- and time-dependent microvascular margination and emigration of neutrophils into the dermis was found (Fig 5*A*). Following coadministration of rIL-1α and cycloheximide, the accumulation of neutrophils was no longer significant (Fig 6), indicating that biosynthesis of a chemotactic protein was responsible for the observed infiltration of neutrophils induced by rIL-1α. Intradermal injection of rIL-8 at concentrations of 0.5 and 5 nM was accompanied by a marked, circumscribed erythema that was evident as early as 2 h after injection. Microscopically, the response to rIL-8 at both concentrations was qualitatively similar to rIL-1α, but both the number and intensity of the predominantly perivascular infiltrates of neutrophils was much greater (Fig 5*B* and Fig 7). As shown by quantitative determination of the neutrophil marker enzyme—myeloperoxidase—in the skin, the inflammatory reaction induced by rIL-8 was also significantly greater than observed with LTB_4 and PAF at any time following injection of different concentrations (4 h response to 10 nM rIL-8, 100 nM LTB_4, and PAF shown in Table II). LTB_4 elicited responses that were quantitatively similar to rIL-1α, but significantly greater than PAF.

DISCUSSION

IL-8, LTB_4, and PAF are known to be present in inflamed human skin in concentrations up to the nanomolar range in certain dermatoses [13,24–26]. In humans, these mediators have been shown to attract primarily neutrophils in vivo [15,27,28], and they probably represent the most important cell-derived neutrophil chemotactic factors present in the skin during inflammation. We have compared the chemotactic and cell aggregating activities of the two lipid mediators and rIL-8 using canine neutrophils as target cells and human neutrophils as reference cells. The potency of rIL-8 was by far the greatest in both assays, potency ratios between rIL-8 and the lipid mediators ranging from approximately 20 to 1000 depending on the assay and the species.

The observation that rIL-8 acts as a potent and persistent stimulant of neutrophil aggregation, also termed homotypic cell adhesion, has not been made previously. This type of homotypic cell adhesion correlates with ligand-induced receptor activation [29] and depends, in contrast to heterotypic adhesion between neutrophils and endothelial cells, solely on the activation of the adhesive surface glycoprotein $CD11_b/CD18$ [30]. The aggregatory neutrophil response to rIL-8 is thus a functional correlate to the recent observation that IL-8 enhances the binding activity of $CD11_b/CD18$ on human neutrophils [31]. The present findings are also in accord with a previous study on human neutrophils that has documented the presence of IL-8 receptors with a higher affinity than reported for other chemotactic factors, including PAF and LTB_4 [32].

An intriguing observation was made in the canine chemotaxis assay. At the optimal concentration, 10 nM, the migration induced by rIL-8 was significantly greater ($p < 0.05$) than that observed towards any concentration of the lipid mediators. Because a recent study dealing with LTB_4, C_{5a}, PAF, formylmethionyl peptide, and LTD_4 revealed that directed migration of canine neutrophils towards LTB_4 was unsurpassed in potency [33], the chemotactic potency and efficacy of rIL-8 in this species is evident. This in vitro

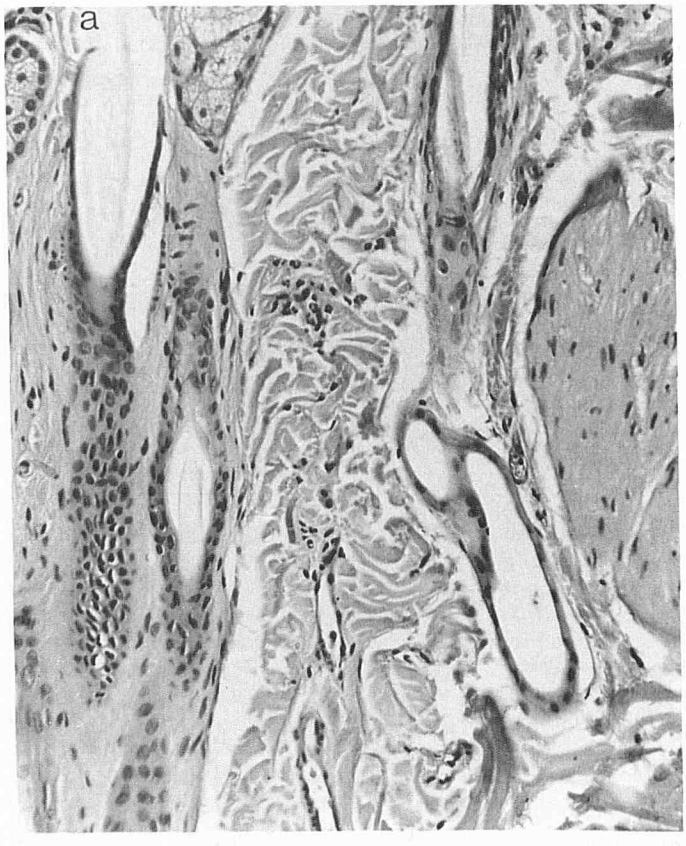

Figure 5. The histologic appearance of canine skin 4 h after injection of 250 I.U. rIL-1α per ml (*a*; score 1 response) or 5 nM rIL-8 (*b*; score 3 response). In both instances, the cellular infiltrate was mainly in a perivascular location, and consisted exclusively of neutrophils. Hematoxylin-eosin × 100.

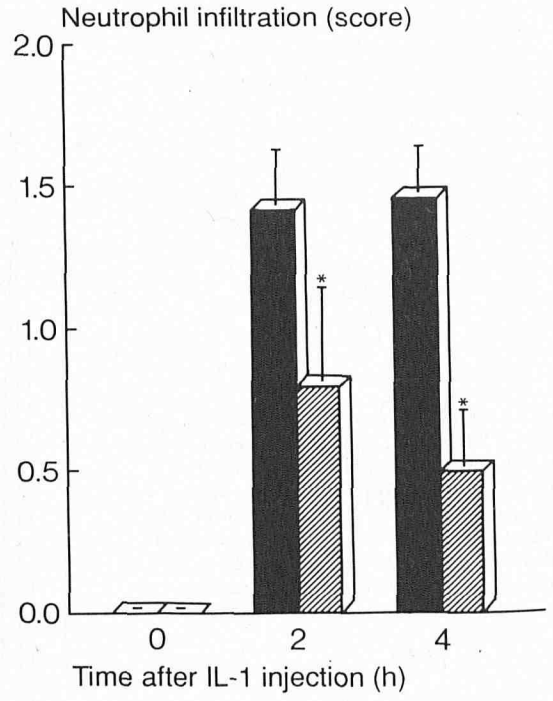

Figure 6. Neutrophil infiltration following intradermal injection in dogs of 250 I.U. rIL-1α per ml with *(shaded)* or without *(black)* 0.1 μmol cycloheximide. Neutrophil infiltration was quantified as follows [22]: 0, normal skin; 1, mild; 2, modest; and 3, pronounced infiltration. Results are presented as the means ± SE of three experiments in which the response was corrected for sham injection sites in each case.

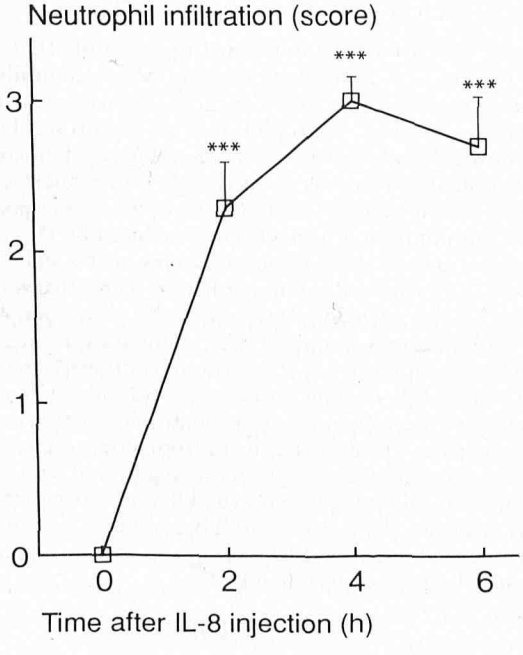

Figure 7. Neutrophil infiltration following intradermal injection in dogs of 5 nM rIL-8. Cellular infiltration was quantified as described for Fig 5. Results are presented as the mean ± SE of three individual experiments.

Table II. Dermal Neutrophil Infiltration Following Injection of 10 nM IL-8, 100 nM LTB_4, and 100 nM PAF

	Sham Sites	rIL-8	LTB_4	PAF
Cells[a]	2.06 ± 0.71	17.50 ± 3.26	7.56 ± 0.79	3.50 ± 0.63
Score	0	3	1–2	0–1

[a] $\times 10^6$ per cm^2.

feature of canine neutrophils, which was also evident in vivo, may in part explain the observation that accumulation of neutrophils at cutaneous cell-mediated immuno-inflammatory sites is much more pronounced in the canine than in the human organism [34–36]. The canine intradermal rIL-8 experiments were in support of this hypothesis, because massive and selective neutrophil infiltration occurred and by far exceeded the infiltration seen following injection of classical lipid and peptide chemoattractants. Potent in vivo action of IL-8 has previously been reported in the skin of rats [6], rabbits [16], and mice [17].

With respect to the modulatory actions of IL-8 on inflammation, a recent report has shown that endothelial IL-8 inhibits adhesion between leukocytes and endothelium, suggesting an anti-inflammatory role for this mediator in certain situations [37]. We propose, however, that IL-8 acting from the tissue side of the microcirculation causes severe inflammation in part by inducing production of LTB_4 by neutrophils, as shown previously [10] and in the present study. The amounts of LTB_4 found in the present in vitro experiments are clearly sufficient to promote neutrophil-endothelial interactions in vitro [38] and in vivo [39] through an effect on both neutrophils and endothelial cells [38]. Due to the rapid tissue clearance of LTB_4, it has in fact been speculated [40] that the most important site at which LTB_4 exerts its pro-inflammatory action is the vascular endothelium where LTB_4 rapidly and potently causes adhesion between endothelial cells and neutrophils [38,39]. Following the adhesive interaction, the neutrophils then migrate towards chemoattractants with a longer tissue half-life, e.g., IL-8.

Because IL-8 has been shown to be the main neutrophil-chemotactic cytokine induced by IL-1 [41,42], we also studied in vivo the indirect chemoattractant response to rIL-1α as a measure of the likely contribution of IL-8 to the overall response. By blocking biosynthesis of proteins with cycloheximide, we observed that significant cell infiltration no longer occurred, indicating that biosynthesis of a chemotactic protein is of prime importance in the canine cutaneous response to rIL-1α. This finding is in agreement with several lines of evidence which suggest that the in vivo chemoattractant properties of rIL-1 are mediated indirectly through IL-8 [5,12,20,41,42].

Present and previous findings support the notion that IL-8, released from sites of injury, immune-mediated reactions and microbial infection, may contribute to the formation of inflammation in the canine and human organism by activating neutrophil functions, including migration, aggregation and LTB_4 production. IL-8 may thus become the target for future pharmacologic intervention, aimed at inhibiting either biosynthesis of, or receptor activation by, IL-8. With respect to the former, we have recently shown that a novel inhibitor of leukotriene biosynthesis, ETH615,* potently inhibits mRNA expression of IL-8 in various cell types [43]. This compound further inhibits zymosan-induced cutaneous inflammation [44], which is in part cytokine-mediated [20], thus supporting the hypothesis that IL-8 is an important mediator of inflammatory reactions in the skin. The present experiments together with the fact that the mediator profile encountered in inflamed canine skin resembles that of humans [44] suggest that the dog may serve as an appropriate model for development of anti-inflammatory drugs with a novel mode of action.

* Kirstein D, Thomsen MK, Ahnfelt-Rønne I: Inhibition of leukotriene biosynthesis and polymorphonuclear leukocyte functions by orally active quinolylmethoxyphenylamines. Pharmacol Toxicol (submitted).

In conclusion, we have described rIL-8 as a potent activator of canine neutrophil aggregation, migration and LTB_4 biosynthesis, suggesting the involvement of this novel cytokine in canine immune-inflammation.

We gratefully acknowledge the excellent technical assistance of Ms. S. Baumgarten, B. Nielsen, S. H. Pedersen and K. Thuneberg, and Mr. H. J. Andersen.

REFERENCES

1. Luger TA, Charon JA, Oppenheim JJ: Heterogeneity of chemoattractant activity for neutrophils and mononuclear cells of ETAF and IL-1. J Immunol 131:187–192, 1983
2. Sauder DN, Mounessa NL, Katz SI, Gallin JI: Chemotactic cytokines: the role of leukocyte pyrogen and epidermal cell thymocyte activating factor in neutrophil chemotaxis. J Immunol 132:828–832, 1984
3. Yoshimura T, Matsushima K, Oppenheim JJ, Leonard EJ: Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear leukocytes: partial characterization and separation from interleukin 1 (IL 1). J Immunol 139:788–793, 1987
4. Thomsen MK: Is interleukin-1α a direct activator of neutrophil migration and phagocytosis in the dog? Agents Actions 29:35–36, 1990
5. Matsushima K, Morishita K, Yoshimura T, Lavu S, Kobayashi Y, Lew W, Appella E, Kung HF, Leonard EJ, Oppenheim JJ: Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J Exp Med 167:1883–1893, 1988
6. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K: The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. Science 243:1464–1466, 1989
7. Thelen M, Peveri P, Kernen P, von Tscharner V, Walz A, Baggiolini M: Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. FASEB J 2:2702–2706, 1988
8. Schröder JM, Mrowietz U, Morita E, Christophers E: Purification and partial characterization of a human monocyte-derived neutrophil-activating peptide that lacks interleukin-1 activity. J Immunol 139:3474–3483, 1987
9. Peveri P, Walz A, Dewald B, Baggiolini M: A novel neutrophil-activating factor produced by human mononuclear phagocytes. J Exp Med 167:1547–1559, 1988
10. Schröder JM: The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates human arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. J Exp Med 170:847–863, 1989
11. Dahinden CA, Kurimoto Y, de Weck AL, Lindley I, Dewald B, Baggiolini M: The neutrophil-activating peptide NAF/NAP-1 induces histamine and leukotriene release by interleukin 3-primed basophils. J Exp Med 170:1787–1792, 1989
12. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K: Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. Immunology 68:31–36, 1989
13. Schröder JM, Christophers E: Identification of C5a des arg and an anionic neutrophil-activating peptide (ANAP) in psoriatic scales. J Invest Dermatol 87:53–58, 1986
14. Seitz M, Dewald B, Gerber N, Baggiolini M: Enhanced release of neutrophil-activating peptide 1/interleukin 8 (NAP-1/IL-8) from mononuclear phagocytes in patients with rheumatoid arthritis—induction and regulation by immune complexes and cytokines (abstr). Clinical Rheumatol 9:133(A), 1990
15. Leonard EJ, Yoshimura T, Tanaka S, Raffed M: In: Dinarello CA, Kluger M, Powanda M, Oppenheim JJ (eds.). Pathophysiologic and Therapeutic Roles of Cytokines. Alan Liss (in press)
16. Van Damme J, Van Beeumen J, Opdenakker G, Billiau A: A novel NH_2-terminal sequence-characterized human monokine possessing neutrophil chemotactic, skin-reactive, and granulocytosis-promoting activity. J Exp Med 167:1364–1376, 1988
17. Furuta TA, Yamagishi J, Kotani H, Sakamoto F, Fukui T, Matsu Y, Yamada M, Yoshimura T, Larsen CG, Oppenheim JJ, Matsushima

K: Production and characterization of recombinant human neutrophil chemotactic factor. J Biochem 106:436–441, 1989

18. Thomsen MK, Ahnfelt-Rønne I: Inhibition by the LTD_4 antagonist, SR2640, of effects of LTD_4 on canine polymorphonuclear leukocyte functions. Biochem Pharmacol 38:2291–2295, 1989
19. Falk W, Goodwin RH, Leonard EJ: A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. J Immunol Meth 33:239–247, 1980
20. Rampart M, Williams TJ: Evidence that neutrophil accumulation induced by interleukin-1 requires both local protein biosynthesis and neutrophil CD18 antigen expression in vivo. Br J Pharmacol 94:1143–1148, 1988
21. Westwick J, Li SW, Camp RD: Novel neutrophil-stimulating peptides. Immunol Today 10:146–147, 1989
22. Thomsen MK, Thomsen HK: Effects of interleukin-1α on migration of canine neutrophils in vitro and in vivo. Vet Immunol Immunopathol 26:385–393, 1990
23. Bradley PP, Priebat DA, Christensen RD, Rothstein GR: Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78:206–209, 1982
24. Barr RM, Brain S, Camp RDR, Cilliers J, Greaves MW, Mallet AI, Misch K: Levels of arachidonic acid and its metabolites in the skin in human allergic and irritant contact dermatitis. Br J Dermatol 111:23–28, 1984
25. Michel L, Denizot Y, Thomas Y, Benveniste J, Dubertret L: Release of paf-acether during allergic cutaneous reactions. Lancet II:404, 1988
26. Fogh K, Herlin T, Kragballe K: Eicosanoids in skin of patient with atopic dermatitis. Prostaglandin E_2 and leukotriene B_4 are present in biologically active concentrations. J Allergy Clin Immunol 83:450–455, 1989
27. Camp RDR, Coutts AA, Greaves MW, Kay AB, Walport MJ: Responses of human skin to intradermal injection of leukotrienes C_4, D_4 and B_4. Br J Pharmacol 80:497–450, 1983
28. Michel L, Mencia-Huerta J-M, Benveniste J, Dubertret L: Biologic properties of LTB_4 and paf-acether in vivo in human skin. J Invest Dermatol 88:675–681, 1987
29. Ford-Hutchinson AW, Evans JF: Neutrophil aggregation and chemokinesis assays. Methods Enzymol 162:72–78, 1988
30. Hogg N: The leukocyte integrins. Immunol Today 10:111–114, 1990
31. Detmers PA, Olsen-Egbert E, Peveri P, Walz A, Baggiolini M, Cohn ZA: Neutrophil activating factor (NAF) enhances the binding activity of CD11b/CD18 (abstr). FASEB J 3:A1087, 1989
32. Samanta AK, Oppenheim JJ, Matsushima K: Identification and characterization of specific receptors for monocyte-derived neutrophil chemotactic factor (MDNCF) on human neutrophils. J Exp Med 169:1185–1189, 1989
33. Strøm H, Thomsen MK: Effects of proinflammatory mediators on canine neutrophil chemotaxis and aggregation. Vet Immunol Immunopathol 25:209–217, 1990
34. Thomsen MK, Kristensen F: Contact dermatitis in the dog. A review and a clinical study. Nord Vet Med 38:129–147, 1986
35. Thestrup-Pedersen K, Larsen CG, Rønnevig J: The immunology of contact dermatitis. A review with special reference to the pathophysiology of eczema. Contact Dermatitis 20:81–92, 1989
36. Thomsen MK, Thomsen HK: Histopathological changes in canine allergic contact dermatitis. A study on spontaneously hypersensitive dogs. Acta Vet Scand 30:379–384, 1989
37. Gimbrone MA, Obin MS, Brock AF, Luis EA, Hass PE, Hébert CA, Yip YK, Leung DW, Lowe DG, Kohr WJ, Darbonne WC, Bechtol KB, Baker JB: Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. Science 246:1601–1603, 1989
38. Lindström P, Lerner R, Palmblad J, Patarroyo M: Rapid adhesive responses of endothelial cells and of neutrophils induced by leukotriene B_4 are mediated by leukocytic adhesion protein CD18. Scand J Immunol 31:737–744, 1990
39. Dahlén, S-E, Björk J, Hedqvist P, Arfors K-E, Hammarström S, Lindgren JÅ, Samuelsson B: Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. Proc Natl Acad Sci USA 78:3887–3892, 1981
40. McMillan RM, Foster SJ: Leukotriene B_4 and inflammatory disease. Agents Actions 24:114–119, 1988
41. Oppenheim JJ, Matsushima K, Yoshimura T, Leonard EJ, Neta R: Relationship between interleukin 1 (IL1), tumor necrosis factor (TNF) and a neutrophil attracting peptide (NAP-1). Agents Actions: 134–140, 1989
42. Van Damme J, Schaafsma MR, Conings R, Lenaerts J-P, Put W, Billau A, Fibbe WE: Interleukin 1 induces different cytokines in human fibroblasts. Lymphokine Res 8:289–292, 1989
43. Larsen CG, Paludan K, Kristensen M, Deleuran B, Thomsen MK, Jørgensen P, Pedersen FS, Matsushima K, Thestrup-Pedersen K: ETH615, a novel inhibitor of interleukin-8 (IL-8) production (abstr). 20th Meeting of the European Society for Dermatological Research, Turin, June 9–12, 1990
44. Thomsen MK, Skak-Nielsen T, Kirstein D, Ahnfelt-Rønne I: Zymosan and arachidonic acid-induced neutrophil infiltration in canine skin: modulation by inhibitors of arachidonic acid metabolism. 7th International Conference on Prostaglandins and Related Compounds, Florence, May 28–June 1, 1990: 232 (A)